Video Article

Safety Precautions and Operating Procedures in an (A)BSL-4 Laboratory: 4. Medical Imaging Procedures

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Abstract

Medical imaging using animal models for human diseases has been utilized for decades; however, until recently, medical imaging of diseases induced by high-consequence pathogens has not been possible. In 2014, the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Integrated Research Facility at Fort Detrick opened an Animal Biosafety Level 4 (ABSL-4) facility to assess the clinical course and pathology of infectious diseases in experimentally infected animals. Multiple imaging modalities including computed tomography (CT), magnetic resonance imaging, positron emission tomography, and single photon emission computed tomography are available to researchers for these evaluations. The focus of this article is to describe the workflow for safely obtaining a CT image of a live guinea pig in an ABSL-4 facility. These procedures include animal handling, anesthesia, and preparing and monitoring the animal until recovery from sedation. We will also discuss preparing the imaging equipment, performing quality checks, communication methods from "hot side" (containing pathogens) to "cold side," and moving the animal from the holding room to the imaging suite.

Video Link

The video component of this article can be found at https://www.jove.com/video/53601/

Introduction

The mission of the National Institute of Allergy and Infectious Diseases (NIAID) Integrated Research Facility at Fort Detrick in Frederick MD (IRF-Frederick) is to perform emerging infectious disease research to understand the clinical disease processes that correlate with the severity of microbial-induced disease. The IRF-Frederick has a unique capability to perform medical imaging in animal models of high-consequence pathogens in an ABSL-4 laboratory[1]. The imaging modalities available to investigators include: computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon computed tomography (SPECT), ultrasound, X-ray, and fluoroscopy. Researchers use available imaging capabilities to monitor disease progression and evaluate efficacy of interventions, such as drug treatment and vaccination, in longitudinal studies.

The imaging modalities at the IRF-Frederick were specifically designed to keep the core components of the equipment outside of high containment[2,3] and accessible for maintenance and repair. This design separates the imaging suite into "hot" (containing pathogen) and "cold sides." To achieve this separation, specially designed tubes were constructed to extend high-containment space into the bores of each imaging modality (**Figure 1**). In addition to providing biological containment, these tubes protect the imaging equipment from gases and chemicals used to decontaminate the high-containment laboratory. Imaging scientists and technologists operate the scanners from the "cold side" while Comparative Medicine (CM) staff handle and monitor animals on the "hot side". Since the CM staff must work closely with imaging scientists to coordinate these experiments, this separation can result in communication challenges.

After evaluating options available, CM staff were outfitted with Bluetooth ear pieces that transmit short-wavelength ultra-high frequency radio waves to phones used to call the imaging staff outside of containment. Due to the design of the facility, wireless access points had to be installed in each of the rooms to overcome signal interference caused by the layers of cement and steel between the "hot" and "cold sides". Thus, communication between CM staff wearing noisy positive-pressure suits and imaging staff outside high-containment is now reliable. Cameras have also been installed on the hot side of the imaging rooms for imaging staff to see activity on the "hot side". With the cameras, the imaging staff can guide CM technicians with animal positioning or any last minute changes to the imaging protocol.

All work in the IRF-Frederick ABSL-4 suit laboratory requires staff to wear positive-pressure encapsulating suits[4]. Wearing these suits reduces mobility, and the heavy latex gloves attached to the suit plus up to three additional layers of gloves compromises dexterity. The result is that procedures take longer to complete and tasks that require fine motor skill are much more difficult. As the biosafety level increases, animal

handling and manipulations become more challenging and time consuming, particularly with small animals. Procedures in an ABSL-4 laboratory can take up to 2-3 times longer than an ABSL-2 laboratory.

The purpose of this article is to visually demonstrate the challenges associated with imaging animal models in an ABSL-4 environment using CT scan procedure of a guinea pig as an example.

Protocol

This protocol adheres to the following animal care guidelines. Animals were housed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All experimental procedures were approved by the National Institute of Allergy and Infectious Diseases, Division of Clinical Research, Animal Care and Use Committee and were in compliance with the Animal Welfare Act regulations, Public Health Service policy, and the *Guide for the Care and Use of Laboratory Animals* recommendations.

1. Prepare CT Scanner (on "Cold Side")

1. Condition the X-Ray Tube
    1. Verify that no staff member is in the scan room and the subject table is empty.
    2. Run the tube conditioning procedure in accordance with the scanner manufacturer's specifications.

2. Perform Air Calibration
    1. Make sure that no objects are in the scanning field.
    2. Perform the air calibration procedure in accordance with the scanner manufacturer's specifications. On many platforms, this calibration is auto- or semi-automatic.

3. Perform Quality Control Scan (on "Hot Side")
    1. Complete the ABSL-4 suit laboratory entry procedures (outlined in detail in reference[5]) with the addition of an extra step. If procedures involve exposure to radioactive materials, clip a dosimeter to the scrubs under the protective suit. Turn in the dosimeter every 2 months for calculation of the radiation exposure.
    2. Place the CT head and body phantom, which is made out of air, water and Teflon, in the appropriate holder on the scanning table.

4. Perform Quality Control Scan (on "Cold Side")
    1. From the cold side, advance the phantom into the center of the bore[6].
    2. Acquire a scan using laboratory standardized quality control (QC) parameters to ensure the scanner is within manufacturer's specifications.
    3. Perform QC computerized analysis of images to calculate mean and standard deviations of signal intensity within regions of interest centered over each material type (air, water or Teflon) according to manufacturer's protocol.
    4. Notify CM staff that the CT scanner is ready for use.
    5. Verify the animal information (identification number, weight, date of birth) with the CM staff.

5. Register Subject Information into the Patient Database.
    1. At the CT scanner console (on the "cold side"), access the scanner's Radiology Information System (RIS). Check that the RIS is populated with the details of the scanning schedule.
    2. Select the current subject and demographics such as identification number, age, date of birth, and exam date; then, the exam type should automatically populate.
    3. Manually enter subject weight.
    4. Select correct subject orientation in CT gantry (*e.g.*, head first, feet first, prone, supine).

2. Prepare Work Areas in the ABSL-4 Suit Laboratory

1. Prepare the Class II Biosafety Cabinet (BSC) or Downdraft Table in the Animal Procedure Room. Turn on BSC (or Downdraft Table) at Least 10 Min Prior to Use.
    1. Clean and disinfect interior surfaces of the BSC or the top surface of the downdraft table using an approved disinfectant such as 5% dual quaternary ammonium (n-alkyl- dimethyl benzyl ammonium chloride, n-alkyl dimethyl ethyl benzyl ammonium chloride) disinfectant solution. Spray interior surfaces of the BSC or the top surface of the downdraft table and wipe surfaces dry after 10 min of contact time. Spray and wipe surfaces with 70% ethanol to remove residual disinfectant.
    2. Place equipment and supplies needed in the BSC or downdraft table, including the anesthesia induction box and animal handling gloves.

2. Prepare the CT Scanner Bed.
    1. Set-up the animal holding device on the CT bed.
    2. Set-up the vital signs monitor to detect heart rate and oxygen saturation. If scanning session is longer than 10 min, monitor temperature.

3. Prepare the Anesthesia Machine.
    1. Check the isoflurane volume in the vaporizer and add more isoflurane if needed.
    2. Perform a leak test by pressurizing the anesthetic breathing circuit with oxygen, checking for escaped gas, and visually inspecting the anesthesia machine for leaks[1].

1. If a leak is detected, determine the source, correct the problem, and perform a follow-up leak test to verify corrections were properly implemented.

3. Weigh the disposable scavenging canister that captures waste anesthetic gas. If the canister is ≥50 g over the initial weight of the canister, replace canister[7].

3. Animal Transport from Animal Procedure Room and Preparation of Anesthesia Induction in CT Scanner Room (on "Hot Side")

1. Transfer the Cage Housing the Guinea Pig from One of the Rodent Holding Rooms Adjacent to the Imaging Suite into the Class II BSC.
   1. Verify the animal ID.
   2. Remove the cage card and keep with the animal.
2. Open the lid to the cage and don animal protective leather gloves over the positive-pressure suit gloves. Gently pick up the guinea pig, place animal in the anesthesia induction box, and cover the box with the lid.
3. Remove the anesthesia induction box containing the animal from the Class II BSC and place it on a transport cart.
4. Using the cart, take the animal to the CT scanner room.
5. In the CT scanner room, immediately connect the scavenging canister and anesthesia machine to the induction box. Turn on the oxygen gas to the induction box and set the vaporizer to deliver 4% isoflurane for initial induction of anesthesia[8].
6. Monitor the animal during anesthesia induction for adequate depth of anesthesia (*e.g.*, unresponsive to external stimuli, muscular tone, stable respiratory and heart rates)[8].
7. Turn off the anesthesia to the induction box when the guinea pig is fully anesthetized.

4. Arrange Subject on Imaging Bed in the CT Scanner (on "Hot side")

1. Remove the Anesthetized Guinea Pig from the Induction Box and Place on the Imaging Bed.
   1. Place the guinea pig on the holding cushion in a prone position.
   2. Apply ophthalmic ointment in both eyes to protect the corneal epithelium from drying out.
2. Administer Maintenance Isoflurane Anesthesia.
   1. Place the nose cone on the guinea pig and turn on gas to deliver 1 L/min of oxygen to the nose cone[8].
   2. Set the vaporizer to deliver 2-3% of isoflurane to the guinea pig via the nose cone.
   3. Once the subject has reached the desired plane of anesthesia (no movement, ~60 breaths per min), reduce the vaporizer setting to deliver 1.0-1.5% of isoflurane for maintenance of anesthesia.
   4. Monitor vital signs including body temperature, heart rate, and respiratory rate. If respiratory rate begins to accelerate or slow down, increase or decrease the percent of isoflurane, respectively[9].
3. Provide an additional heat source to maintain guinea pig body temperature between 37 and 39 °C, if necessary, depending on the expected length of data acquisition and depth of anesthesia maintained[8,9].
4. Secure a plastic cover over the top of the holding cushion.
5. Advance imaging bed into containment tube.

5. Setting the Image Field of View

1. Activate the laser system (imaging staff) on the "cold side" of the CT scanner room to position the guinea pig for CT scanning. Use the table "in" and "out" buttons to cover the anatomy of interest under the laser crosshairs.
2. To set the field of view, use the laser button to set the starting point over the anatomy of interest.
3. Contact CM staff on "hot side" to place a lead shield between themselves and the CT scanner.

6. Acquire Images

1. Acquire survey scan for slice placement for CT study images according to manufacturer's protocol.
2. Prescribe slice placement for CT study images on survey images.
3. Coordinate with "hot-side" personnel if contrast injection is to be used.
4. Acquire study scan according to manufacturer's protocol.
5. Reconstruct CT images at the scanner console according to manufacturer's protocol.
6. Send images to a picture archiving and communication system. Perform further qualitative and quantitative analyses from this archiving system according to manufacturer's protocol.

7. Post-scan Recovery

1. Transfer guinea pig from scanner bed to a clean microisolator cage with food and treats.
2. Using the cart, transport the cage with animal back to its housing area.
3. Monitor the animal until fully recovered from anesthesia.
4. Once fully recovered, return the cage to the high-efficiency particulate air (HEPA)-filtered ventilated racks.

8. Disinfection of Scanner Bay and Equipment

1. Disinfect the scanner bay area (*e.g.*, surfaces that were in direct contact with the animal, scanner bed, scanner room floor, door handles) with a 5% dual quaternary ammonium solution for a contact time of 10 min.
2. Rinse surfaces with a 70% ethanol solution after a 10 min exposure to dual quaternary ammonium solution.
3. Wipe items that cannot be sprayed directly with dual quaternary ammonium solution (sensitive electronic equipment) with a dual-quaternary-ammonium-saturated cloth followed by a ethanol-saturated cloth.

Representative Results

Strict adherence to all safety procedures and standard operating procedures for animal handling is essential for working safely in an ABSL-4 laboratory. Transferring infected animals within the induction box from the animal procedure room to the imaging suite minimizes the risk of contamination of common corridors. By following the procedures required, no laboratory-acquired infections or cross-contamination of animal subjects has been recorded while conducting ABSL-4 research at the IRF-Frederick.

ABSL-4 viral pathogens can induce lung-related pathologies[10,11], and CT is a valuable tool for monitoring disease progression in animal models of infection. CT images are created from the absorption of x-ray radiation into tissues, and imaging experiments can be performed relatively quickly, minimizing the amount of anesthesia required. In CT, the greater the density of the object imaged, such as bone, the brighter the object appears (**Figure 2**). Density of organ tissues tend to be of intermediate intensity (gray), while air-filled tissues such as healthy lungs, appear black. CT acquires multiple radiographs over a 360° view that is reconstructed into a 3-dimensional view of the body. For greater delineation between organs and infectious disease-related pathologies, intravenous contrast agents may be used.

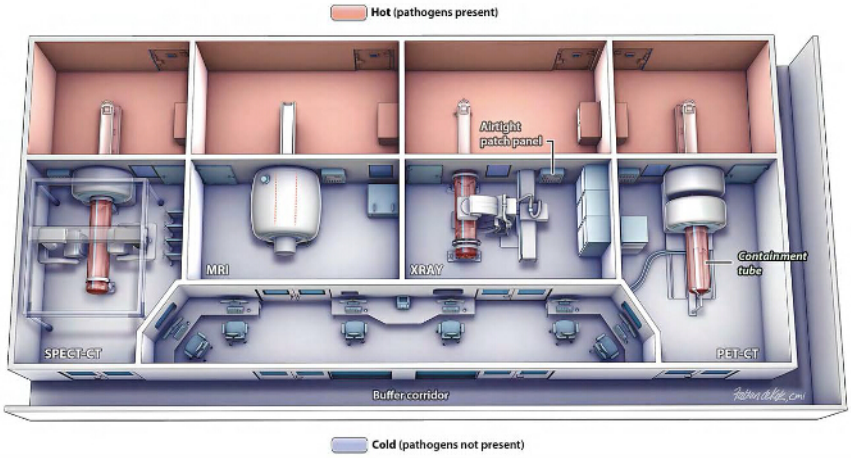

Figure 1: High containment design of imaging modalities at the IRF-Frederick. This design separates the imaging suite into "hot" and "cold" sides. Specially designed tubes extend the high containment space of the "hot side" into the bores of each scanner on the "cold side". Imaging scientists and technologists operate the scanners from the "cold side" while Comparative Medicine (CM) staff handle and monitor animals on the "hot side". Please click here to view a larger version of this figure.

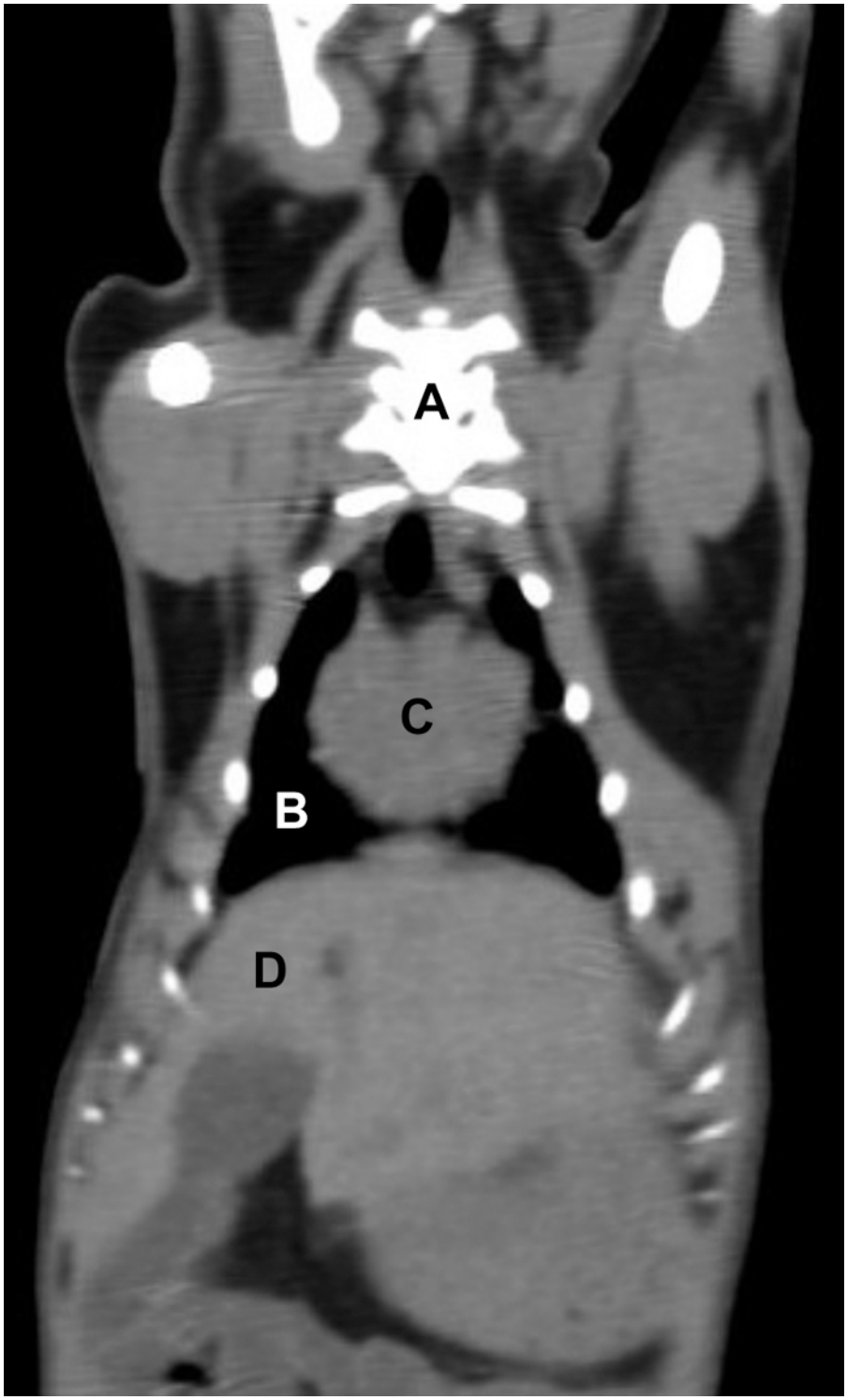

Figure 2: CT image of the upper torso of a guinea pig. X-ray absorption is greater in bone, therefore bone (**A**) will appear brightest in the image, and air (**B**, lung tissue) will appear darkest, and soft tissue (**C**: heart, **D**: liver) will appear intermediate in contrast (A)BSL-4 viral pathogens induce lung-related pathology, making CT a valuable tool for monitoring disease progression in animal models of infection. Please click here to view a larger version of this figure.

Discussion

Previous articles in this series have emphasized the extensive training, attention to detail, safety procedures, and additional engineering controls required to work safely in a maximum containment laboratory[12,13]. Performing work safely is the highest priority in these laboratories. This philosophy is even more important when working with live animals due to additional hazards such as the potential for infected animals to inflict bites or scratches or to generate aerosols[7]. These procedures emphasize the safe handling and transport of animals from the holding room

to the imaging rooms. Animal manipulations and monitoring during anesthesia are demonstrated to give the viewer a realistic portrayal of the attention to detail and safety considerations required while working in an ABSL-4 laboratory.

We describe a protocol to perform non-invasive medical imaging in an ABSL-4 laboratory using a CT scan of a guinea pig as an example. Several steps are critical to successful imaging in the BSL-4 laboratory. The first critical step includes verifying that safety checklists are completed to ensure all safety systems are working properly before entry into the lab[5]. Staff must follow proper entry and exit procedures to ensure they are working safely in the BSL-4 laboratory. The second critical step is to verify that staff are either not present in the scan room or they are behind a portable lead shield during the conditioning of the x-ray tube and any subsequent scans. It is important to determine that the CT scanner is ready and working properly before anesthetizing and preparing animals for imaging. The next critical step is to perform a quality control scan with an appropriate phantom and notify animal technicians when the CT scanner is operational and ready for use. Clear and effective communication is also important to execute imaging protocols as animal handling staff are physically separated from imaging technologists operating the scanners on the "cold side".

Medical imaging in a BSL-4 laboratory is a challenging procedure as all animal handling procedures must be done while wearing the positive pressure suit and multiple pairs of gloves, including heavy suit gloves. Basic animal handling procedures are modified to fit the safety considerations of the BSL-4 laboratory. Handling of awake animals is minimized and modified to reduce chances of bites or scratches. For example, leather work-gloves are worn to protect suit gloves when picking up awake guinea pigs and other larger rodents. Mice are only handled with forceps and must be anesthetized first before scruffing them for intraperitoneal injections. Injection techniques may need to be modified to ensure a higher degree of safety. Forceps and/or restraint devices are used to perform injections in rodents rather than using hand restraint alone. The complexity and time needed to perform imaging depends on several factors, including the modality chosen and the species used. Limitations to performing CT scans include the difficulty in administering contrast agents to certain laboratory animal species. Guinea pigs, in particular, do not have easily accessible veins for administering a contrast agent intravenously. Difficulty with this administration is compounded by difficulty of fine motor manipulation while wearing personal protective equipment. Additionally, the timing of contrast agent injections must be coordinated with staff on the "cold side" that are operating the scanner.

The unique design of the imaging facility creates challenges that require modifications of imaging techniques. One challenge was the difficulty with communication due to the physical separation of imaging technologists who run the scanning equipment and animal technicians handling and monitoring the animals. Bluetooth phones with headsets worn inside the suit are used to communicate with imaging technologists to coordinate and perform the scans. If this communication method fails, hand-written message on white boards can be displayed through laboratory windows. The design of each imaging modality includes a special tube which extends the high containment facility into the bore of each scanner. After each imaging subject is positioned on an imaging bed, the subjects are a greater distance from and less visible to the technicians who monitor them while under anesthesia. Longer anesthesia circuits, monitoring cables, and infusion lines are required in this design.

The IRF-Frederick has the capability to perform biomedical imaging in a variety of laboratory animals from mice to nonhuman primates. CT can be used to monitor disease progression in a variety of animal models of infectious diseases. Evaluating the efficacy of potential therapeutic interventions, including vaccines, and identifying biomarkers of disease processes are future applications of this technique.

Disclosures

The authors have nothing to disclose.

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